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Development of COVID-19 vaccines utilizing gene therapy technology

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REVIEW

There is currently an outbreak of respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Coronavirus disease 2019 (COVID-19) is caused by infection with SARS-CoV-2. Individuals with COVID-19 have symptoms that are usually asymptomatic or mild in most initial cases. However, in some cases, moderate and severe symptoms have been observed with pneumonia. Many companies are developing COVID-19 vaccine candidates using different technologies that are classified into four groups (intact target viruses, proteins, viral vectors and nucleic acids). For rapid development, RNA vaccines and adenovirus vector vaccines have been urgently approved, and their injection has already started across the world. These types of vaccine technologies have been developed over more than 20 years using translational research for use against cancer or diseases caused by genetic disorders but the COVID-19 vaccines are the first licensed drugs to prevent infectious diseases using RNA vaccine technology. Although these vaccines are highly effective in preventing COVID-19 for a short period, safety and efficiency evaluations should be continuously monitored over a long time period. As the time of writing, more than 10 projects are now in phase 3 to evaluate the prevention of infection in double-blind studies. Hopefully, several projects may be approved to ensure high-efficiency and safe vaccines.

Keywords: gene therapy, SARS-CoV-2, virus vector

Introduction

There is currently an outbreak of respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Coronavirus disease 2019 (COVID-19) is caused by infection with SARS-CoV-2. To combat the worldwide COVID-19 pandemic, the development of effective and safe vaccines against SARS-CoV-2 is urgently required. Many pharmaceutical companies and researchers are developing vaccines against SARS-CoV-2, including vaccines based on inactivated target virus or viral proteins, adenovirus vectors and RNA/DNA from SARS-CoV-2 (1–5). The target antigen is the spike glycoprotein of SARS-CoV-2 or its receptor-binding domain (RBD), which is essential for virus entry into cells (6). The vaccines are classified into four groups based on the vaccine technology.

Classification based on the vaccine technology

Intact target virus vaccines

Intact target virus vaccines include inactivated and attenuated viruses, which are common as preventive vaccines for infectious diseases. Basically, this type of vaccine exposes the body to viruses that do not cause disease but induce an efficient immune response that can inhibit viral infection. These viruses are manufactured through passage in human cells or animals, which may cause favorable mutations for use in weakened or inactivated vaccines. Recently, it has become possible to alter the genetic code to make favorable mutations for weakened or inactivated vaccines. A Chinese company has rapidly developed an inactivated-virus vaccine for SARS-CoV-2 (7) and performed phase 3 clinical trials. They announced that the vaccine had an efficiency of 79% (not yet published), and it has been approved in China and the United Arab Emirates. An Indian company also rapidly developed an inactivated-virus vaccine (8) and performed phase 3 clinical trials (not yet published). It has been approved under an emergency authorization in India.

Protein-based vaccines

This type of vaccine includes whole proteins, a fragment of a protein or an outer shell that mimics the coronavirus, such as virus-like particles (VLPs). This approach tries to synthesize only the necessary part of the protein as an antigen, such as spike glycoprotein or its RBD. Importantly, this vaccine type may require the co-administration of adjuvants to

stimulate innate immunity, leading to the activation of an efficient acquired immune response. Novavax has reported having promising results in phase 1/2 clinical trials using recombinant protein and adjuvants (9). Their vaccine, NVX-CoV2373, is a recombinant nanoparticle vaccine composed of trimeric full-length SARS-CoV-2 spike glycoproteins and adjuvant.

VLPs may induce a strong immune response without infectious risk because they mimic only the shell of the coronavirus structure. However, VLPs are usually difficult to manufacture, especially at a clinical grade on a large scale.

Viral vector vaccines

Viral vectors are tools to efficiently deliver genetic material into cells. As viral vectors, viruses are genetically engineered to efficiently produce some coronavirus proteins. Measles or adenovirus have been utilized as vectors that can efficiently enter cells, but the viral vector itself is weakened and cannot cause disease. This type of viral vector has achieved high transfection efficiency and has been developed over more than 20 years, using translational research on gene therapy, as an efficient therapy for cancer or diseases caused by genetic disorders. Recently, an Ebola vaccine using virus vectors has been approved based on a clinical trial (10–13), which is the first approved viral vector vaccine drug for infectious disease.

In the challenge of rapid vaccine development for COVID-19, an adenovirus vector has been utilized, which is a non-replicating viral vector. Because the adenovirus vector is strongly immunogenic, a situation in which the immune system could learn to recognize the viral vector as a foreign object that needs to be destroyed must be avoided. Therefore, researchers at Oxford University, with AstraZeneca, developed a vaccine by genetically engineering an adenovirus that normally infects chimpanzees. Early in the pandemic, they started a phase 1/2 trial. The results showed no severe side effects in the trial, but antibodies against the coronavirus were induced, as well as other immune defenses (14). In the immunogenicity data, the SARS-CoV-2-neutralizing antibody titer (ID50 shown in geometric mean titer [GMT]) was increased in both low dose (2.2×10^{10} viral particles) and standard dose (5×10^{10} particles) and there is no difference at different ages (Table 1). Their group began phase 2/3 trials in the UK and India and later launched phase 3 trials in Brazil, South Africa and the USA (Table 2).

The Oxford University/AstraZeneca clinical trial was halted to investigate a volunteer who developed transverse myelitis. Within 1 week, the trial began again after the judgment of the safety evaluation committee. In a study of the first 131 cases of COVID-19 in trials in the UK and Brazil, the vaccine had good efficacy. Although the volunteers all received two doses, an initial half-strength dose and second standard-dose shots led to 90% efficacy, whereas two standard-dose shots led to 60.3% efficacy in UK and 64.2% efficacy in Brazil (15). This vaccine has been approved for emergency use in the UK, Argentina, Australia, the European Union, India, South Korea, Thailand etc. and the World Health Organization gave approval as an emergency use listing.

Researchers at the Beth Israel Deaconess Medical Center/Johnson and Johnson also utilized adenovirus 26 (Ad26) for a COVID-19 vaccine. They started a phase 1/2 trial in July 2020. The study design was to administer the Ad26.CoV2.S

Table 1. Early clinical trials of adenovirus vector-based COVID-19 vaccines

Vaccine and country	Dose and usage (number of participants and age)	Neutralizing antibody titer (GMT)
AZD1222	Twice at 4-week intervals	
COV001, UK	5×10^{10} viral particles	218
	(487: 18–55 years old)	
COV002, UK	2.2×10^{10} viral particles (low dose)	
	(41: 18–55 years old)	161
	(28: 56–69 years old)	143
	(34: ≥70 years old)	150
COV002, UK	5×10^{10} viral particles (standard dose)	
	(39: 18–55 years old)	193
	(20: 56–69 years old)	144
	(47: ≥70 years old)	161
Ad26.COV2.S, Belgium and USA	Once or twice at 56-day intervals	
	5×10^{10} viral particles (low dose)	
	(162: 18–55 years old)	288 (single), 827 (twice)
	(161: ≥65 years old)	277 (single)
	1×10^{11} viral particles (high dose)	
	(158: 18–55 years old)	488 (single), 1266 (twice)
	(161: ≥65 years old)	212 (single)
CanSino (Ad5), China	Twice at 4-week intervals	
	(18–60 years old)	
	5×10^{10} viral particles (36)	14.5
	1×10^{11} viral particles (36)	16.2
	1.5×10^{11} viral particles (36)	34.0
	5×10^{10} viral particles (129)	55.3
	1×10^{11} viral particles (253)	61.4

Neutralizing antibody titer (ID50) was shown in GMT (geometric mean titer) which was referred from each publication, and the highest value was selected in each study.

Table 2. Late clinical trials of adenovirus vector-based COVID-19 vaccines

Study	Dose and usage (number of participants and age)	Vaccine efficacy (onset/cases)
AZD1222		90.0%
COV002, UK	$2.2 \times 10^{10} + 5 \times 10^{10}$ viral particles	
	(1367: 18–55 years old)	(3/1367)
	Control (1374: 18–55 years old)	(30/1374)
COV002, UK		60.3%
	5×10^{10} viral particles	(15/2377)
	(1879: 18–55 years old) (285: 56–69 years old) (213: ≥70 years old)	
	Control	(38/2430)
	(1922: 18–55 years old) (293: 56–69 years old) (215: ≥70 years old)	
COV003, Brazil		64.2%
	5×10^{10} viral particles	(12/2063)
	(1843: 18–55 years old) (209: 56–69 years old) (11: ≥70 years old)	
	Control	(12/2025)
	(1833: 18–55 years old) (187: 56–69 years old) (5: ≥70 years old)	
COV002 + 003		62.1%
	5×10^{10} viral particles	(27/4440)
	Control	(71/4445)
Ad26.COV2.S		66.1%
	5×10^{10} viral particles	(66/190 306)
	Control	(193/19 178)
USA		72.0%
South Africa		64.0%
Brazil		68.1%

vaccine at a dose of 5×10^{10} particles (low dose) or 1×10^{11} particles (high dose) in a single-dose or two-dose regimen. The results showed no severe side effects in the trial (16). As shown in Table 1, the neutralizing antibody titer was increased at day 57 after the first vaccine dose (GMT: 288−488) for both the low dose (5×10^{10} viral particles) and the high dose (1×10^{11} particles). A second dose provided a further increase in the neutralizing antibody titer (GMT: 827–1266). On the basis of these results, they launched a phase 3 trial using one dose rather than two in the USA, South Africa and Brazil. This single shot vaccine led to 62.1% efficacy in total, 72.0% efficacy in the USA, 64.0% efficacy in South Africa and 68.1% in Brazil. There is no difference across ages (66.1% for patients at 18–59 years old and 66.2% for those ≥60 years old). This vaccine has been approved as for emergency use in the USA, Canada and Bahrain.

Other projects using adenovirus vectors have also currently moved to phase 3 clinical trials, and some vaccines have been rapidly approved in China and Russia. In China, an early-stage trial was quickly conducted using recombinant adenovirus type-5 (Ad5). One hundred and eight participants received a single dose of the vaccine that induced an immune response within 2 weeks (17). In phase 2, the 508 participants were randomly assigned to receive the vaccine (low dose or high dose) or placebo. Both doses of the vaccine induced significant neutralizing antibody responses and T-cell activation (18). On the basis of these results, the Chinese military approved the vaccine on 25 June 2020 as a specially needed drug. This vaccine has also been approved for emergency use in Mexico and Pakistan.

In Russia, a combination vaccine of two adenoviruses, Ad5 and Ad26, has been developed. In the phase 1 and 2 studies, 9 volunteers received Ad26 in phase 1, 9 received Ad5 in phase 1 and 20 received Ad26 and Ad5 in phase 2. The heterologous Ad26 and Ad5 vector-based COVID-19 vaccine has a good safety profile and induces humoral and cellular immune responses in participants (19). In the preliminary evidence based on their phase 3 trial, the vaccine demonstrated more than 90% efficacy with no serious side effects (not yet published). This vaccine has been approved for emergency use in the Argentina, Bahrain, Egypt, Mexico, Vietnam etc. Surprisingly, it has been combined with another adenovirus vector vaccine produced by AstraZeneca. The two teams will evaluate their vaccines to see if the mixture can increase the efficacy of the AstraZeneca vaccine.

Nucleic acid vaccines

Vaccines using virus DNA or RNA are included in this group. On the basis of genomic information, DNA or RNA of the coronavirus gene or a modified gene is delivered into the cells in the body to provoke an immune response. These types of vaccines can be easily developed to use only genetic material, not viruses, and potentially activate cellular immunity as well as humoral immunity. However, one of the problems to overcome for clinical development is the low transfection efficiency of nucleic acids in the body.

BioNTech and Pfizer rapidly began designing vaccines based on messenger RNA (mRNA). RNA is usually fragile and digested into pieces if it is injected directly into the body. Therefore, they modified the nucleic acid and wrapped the mRNA of spike protein in lipid nanoparticles. After injection, the vaccine particles can fuse into cells and release the mRNA. The spike protein can be produced based on the transferred RNA and functions as an antigen.

BioNTech and Pfizer presented the initial results of clinical trials very rapidly (Table 3) (20, 21). Initially, they designed two types of lipid nanoparticle-formulated RNA vaccine candidates: BNT162b1, which encodes a secreted trimerized SARS-CoV-2 RBD, and BNT162b2, which encodes a prefusion stabilized membrane-anchored SARS-CoV-2 full-length spike. In both younger and older adults, the two vaccine candidates elicited similar dose-dependent SARS-CoV-2-neutralizing antibody titers. BNT162b2 was associated with less systemic reactogenicity, particularly in older adults.

These results supported selection of the BNT162b2 vaccine candidate for phase 2/3 large-scale safety and efficacy evaluation (21). In the phase 3 clinical trial, a total of 43 448 participants received injections: 21 720 with BNT162b2 and 21 728 with placebo. There were 8 cases of COVID-19 with onset at least 7 days after the second dose among participants assigned to receive BNT162b2 and 162 cases among those assigned to receive the placebo; BNT162b2 was thus 95% effective in preventing COVID-19 (22). The Food and Drug Administration (FDA) granted it the first approval given

Table 3. Early clinical trials of RNA vaccines for COVID-19

Vaccine and country	Dose and usage (number of participants and age)	Neutralizing antibody titer (GMT)
BNT162b1	Twice at 3-week intervals	
Pfizer	0.01 mg (12: 18–55 years old)	180
	0.03 mg (12: 18–55 years old)	437
	0.1 mg (once) (12: 18–55 years old)	94
BNT162b1	0.01 mg (12: 18–55 years old)	180
	(12: 65–85 years old)	33
	0.02 mg (12: 18–55 years old)	203
	(12: 65–85 years old)	105
	0.03 mg (12: 18–55 years old)	437
	(12: 65–85 years old)	105
BNT162b2	0.01 mg (12: 18–55 years old)	157
	(12: 65–85 years old)	111
	0.02 mg (12: 18–55 years old)	363
	(12: 65–85 years old)	84
	0.03 mg (12: 18–55 years old)	361
	(12: 65–85 years old)	206
mRNA-1273	Twice at 4-week intervals	
Moderna	0.025 mg (15: 18–55 years old)	112.3
	0.1 mg (15: 18–55 years old)	343.8
	0.25 mg (15: 18–55 years old)	332.2
	0.1 mg (15: 56–70 years old)	402
	0.1 mg (15: ≥71 years old)	317
mRNA-1273	Twice at 4-week intervals	
CureVac	0.002 mg (33: 18–60 years old)	48
	0.04 mg (33: 18–60 years old)	40
	0.06 mg (36: 18–60 years old)	20
	0.08 mg (34: 18–60 years old)	57
	0.1 mg (11: 18–60 years old)	113

Neutralizing antibody titer (ID50) was shown in GMT (geometric mean titer) which was referred from each publication, and the highest value was selected in each study.

by the USA to a coronavirus vaccine. A number of other countries around the world have also given emergency approval, and the World Health Organization gave approval as an emergency use listing.

Similarly, Moderna also developed their own vaccine, mRNA-1273, based on RNA vaccine technology. They presented the initial results of a clinical trial in a dose-escalation study (23). In a phase 3 trials, 30 420 volunteers were randomly assigned in a 1:1 ratio to receive either vaccine or placebo (15 210 participants in each group). Symptomatic COVID-19 illness was confirmed in 185 participants in the placebo group (56.5 per 1000 person-years) and in 11 participants in the mRNA-1273 group (3.3 per 1000 person-years); vaccine efficacy was 94.1% (24). The Moderna vaccine is the second one authorized by the FDA, with approval coming 1 week after the vaccine made by Pfizer and BioNTech. This vaccine has been approved as for emergency use in Canada, the European Union, Iceland, Norway, Singapore, UK, USA, Vietnam etc.

Other projects using RNA vaccines have also conducted phase 3 clinical trials in Germany. CureVac also designed an mRNA-Lipid nanoparticle vaccine (CVnCoV) and utilized two doses ranging from 0.002 to 0.012 mg at 28-day intervals in the phase 1 study. The neutralizing antibody titer was increased, especially with the highest dose (0.1 mg) at day 43 (14 days after the second dose) (25). CureVac launched a phase 3 trial with CVnCoV 0.012 mg vaccine in two doses at 28-day intervals, recruiting up to 36 500 volunteers. Although these RNA vaccines are highly effective in preventing COVID-19 in the short term, safety and efficiency evaluations should be continuously monitored for a long time period.

In terms of DNA vaccines, several projects have been conducted for COVID-19 vaccines, but no DNA-based vaccines have been approved up to now. DNA vaccines can be delivered intra-muscularly or intra-dermally, which primarily induces the expression of antigen in myocytes or keratinocytes, respectively, but also in antigen-presenting cells near the injection side (26–30). Thus, DNA vaccines can theoretically activate cellular immunity as well as the humoral immunity, and be stable, cost-efficient, easy to manufacture and safe to handle.

In the history of DNA vaccines, the first clinical trial was started in the 1990s and more than a hundred clinical trials that focus on DNA vaccination have been registered. There are no approved DNA vaccines for use in humans up to now. Nevertheless, some DNA-based vaccines were approved for veterinary use, including a vaccine against West Nile virus in horses (31) and canine melanoma (32). In the first human clinical trials, a DNA vaccine against HIV showed a potential, but not significant, immunogenicity (33). A malaria DNA vaccine successfully induced a CD8 T-cell response after immunization of a mixed plasmid encoding different antigens (34). A DNA vaccine for hepatitis B virus showed the induction of a humoral response in patients (35). However, in most cases, the level of the immune response was not sufficient to elicit significant clinical benefits. The unsolved issue is the low immunogenicity of DNA vaccines in humans, probably because of the low amount of administered plasmid DNA. To overcome this issue, the delivery and transfection system has been optimized over many years (36). Actually, jet injectors (37), liposomes (38, 39) and electroporation (40) have enhanced responses through an increased efficiency of DNA delivery and have been recently utilized in clinical trials.

In the challenge of plasmid DNA vaccines for COVID-19, INOVIO developed a DNA vaccine that is delivered into the skin by electroporation. In a phase 1 trial, they confirmed a humoral and cellular immune response without serious adverse effects (41). Zudus in India also developed a plasmid DNA vaccine that is delivered into the skin by injectors. They have completed a phase 2 trial and started phase 3 trials. We also developed a plasmid DNA vaccine that is delivered into muscle by a needle injection or into the skin by a novel pyro-drive jet injector in a phase 1/2 trial. This delivery system may possibly enable precise targeting with effective gene expression intra-dermally, which will hopefully overcome the remaining issue in the translational research of DNA vaccines in future years.

Guidance for COVID-19 vaccine development

In June 2020, the FDA provided timely guidance to address the COVID-19 public health emergency (42). The FDA is committed to assisting sponsors in the clinical development and licensure of vaccines for the prevention of COVID-19. This guidance has been classified into the following parts: (i) chemistry, manufacturing and controls;

(ii) non-clinical data; (iii) clinical trials; (iv) post-licensure safety evaluation; and (v) diagnostic and serological assays. Here, I briefly summarize the essence of safety evaluation of non-clinical data and clinical studies for COVID-19 vaccine development.

Previous vaccine development against other coronaviruses [SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV)] in animal models has raised a theoretical risk for COVID-19 vaccine-associated enhanced respiratory disease (ERD). In these studies, vaccine constructs against other coronaviruses were administered with the respective wild-type virus. These studies have shown evidence of immunopathologic lung reactions characteristic of T_h2-type hypersensitivity. Similar observations have been shown in studies of formalin-inactivated respiratory syncytial virus (RSV) vaccines upon subsequent challenge with RSV because of natural exposure or in the laboratory (43–47). The current knowledge and understanding of the potential risk of COVID-19 vaccine-associated ERD is limited. On the basis of the above theoretical risk from previous vaccine development against SARS-CoV and MERS-CoV, COVID-19 vaccine candidates should induce high neutralizing antibody titers and T_h1-type T-cell polarization. The post-vaccination challenge test in animal models (i.e. hamster model) can be conducted and evaluated prior to enrolling large numbers of human subjects in phase 2 and 3 clinical trials.

In a clinical trial, the goal of clinical trials should be to achieve direct evidence of vaccine efficacy in protecting humans from SARS-CoV-2 infection. In the trial population, early phase studies may expose 10–100 participants to each vaccine candidate. Participants at high risk of severe COVID-19 or at high risk of SARS-CoV-2 exposure (e.g. healthcare workers) should be excluded from early phase studies. Older adult participants may be enrolled in early phase studies so long as they do not have medical comorbidities associated with an increased risk of severe COVID-19. Preliminary clinical safety and immunogenicity data will be collected for each dose level and age group, such as younger versus older adults or neutralizing versus total antibody responses. Placebo control and blinding are not required for early phase studies.

Initial late-phase trials should be preceded by adequate characterization of safety and immunogenicity (a few hundred participants for each vaccine candidate, dose level and age group). The inclusion of diverse populations should be encouraged in all phases of vaccine clinical development. COVID-19 outcomes from earlier clinical development are also important sources of information to support clinical trials with thousands of participants. The COVID-19 outcomes in individuals with prior SARS-CoV-2 infection, which might have been asymptomatic, are also important to examine because pre-vaccination screening for prior infection is unlikely to occur in practice with the deployment of licensed COVID-19 vaccines. Therefore, COVID-19 vaccine trials need not exclude participants with history or laboratory evidence of prior SARS-CoV-2 infection (Table 4).

Late-phase trials should be randomized, double blinded and placebo controlled. An individually randomized controlled trial with 1:1 randomization between vaccine and placebo groups is usually the most efficient study design for demonstrating vaccine efficacy. An efficacy trial that evaluates multiple vaccine candidates against a single placebo group may be an acceptable approach to further increase efficiency. If the availability of a COVID-19 vaccine proven to be safe and effective precludes ethical inclusion of a placebo control group, that vaccine could serve as the control treatment in a study designed to evaluate efficacy with non-inferiority hypothesis testing. Follow-up of study participants for COVID-19 outcomes (in particular, for severe COVID-19 disease manifestations) should continue as long as feasible, ideally at least 1–2 years. Because a COVID-19 vaccine might also be effective in preventing severe COVID-19, the evaluation of the onset of severe COVID-19 should be considered as a secondary endpoint. For statistical consideration, the efficacy of the primary endpoint for a placebo-controlled trial should be at least 50%.

Table 4. Late clinical trials of RNA vaccine for COVID-19

Study	Dose and usage (number of participants and age)	Vaccine efficacy (onset/cases)
BNT162b BioNTech/ Pfizer	Twice at 3-week intervals 0.03 mg	95.5% (8/18 198)
	Placebo	(162/18 325)
	(18–55 years old)	95.6%
	(>55 years old)	93.7%
	(≥65 years old)	94.7%
	(≥75 years old)	100%
mRNA-1273 Moderna	Twice at 4-week intervals	94.1%
	0.1 mg	(11/14 134)
	Placebo	(185/14 073)
	(18–64 years old)	95.6%
	(≥65 years old)	86.4%

Conclusion

At the time of writing, more than 10 projects are now in phase 3 to evaluate the prevention of infection in double-blind studies. A few vaccines have been approved for full use, and several vaccines have been approved for early or limited use in the world. Hopefully, several projects may be fully approved as high-efficiency and safe vaccines.

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